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For: A NOVEL HAEMOPOIETIN  
RECEPTOR AND GENETIC SEQUENCES  
ENCODING SAME

Dated: November 28, 2000

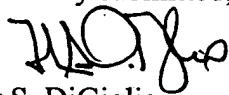
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CLAIM OF PRIORITY

Sir:

Applicants in the above-identified application hereby claim the right of priority in connection with Title 35 U.S.C. § 119 and in support thereof, herewith submits certified copies of Australian Patent Application Nos. PN 7276, filed on December 22, 1995, PN 6135 filed on October 23, 1995 and PO 2208 filed on September 9, 1996.

Respectfully submitted,

  
Frank S. DiGiglio

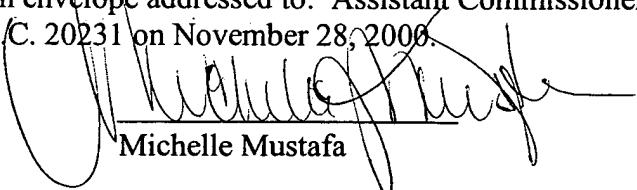
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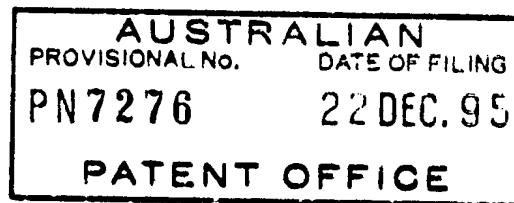
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I, LISA TREVERROW, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PN 7276 for a patent by THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH filed on 22 December 1995.

WITNESS my hand this  
Thirteenth day of November 2000

LISA TREVERROW  
TEAM LEADER EXAMINATION  
SUPPORT AND SALES





THE WALTER AND ELIZA HALL  
INSTITUTE OF MEDICAL  
RESEARCH

A U S T R A L I A  
Patents Act 1990

PROVISIONAL SPECIFICATION  
for the invention entitled:

"A NOVEL HAEMPOIETIN RECEPTOR AND GENETIC SEQUENCES ENCODING  
SAME - II"

The invention is described in the following statement:

**A NOVEL HAEMOPOIETIN RECEPTOR AND GENETIC SEQUENCES  
ENCODING SAME - II**

5

The present invention relates generally to a novel haemopoietin receptor or components or parts thereof and to genetic sequences encoding same. The receptor molecules and their components and/or parts and the genetic sequences encoding same of the present invention are useful in the development of a wide range of agonists, antagonists, 10 therapeutics and diagnostic reagents based on ligand interaction with its receptor.

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined 15 following the bibliography.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the 20 exclusion of any other integer or group of integers.

The rapidly increasing sophistication of recombinant DNA techniques is greatly facilitating research into the medical and allied health fields. Cytokine research is of particular importance, especially as these molecules regulate the proliferation, 25 differentiation and function of a wide variety of cells. Administration of recombinant cytokines or regulating cytokine function and/or synthesis is becoming increasingly the focus of medical research into the treatment of a range of disease conditions.

Despite the discovery of a range of cytokines and other secreted regulators of cell 30 function, comparatively few cytokines are directly used or targeted in therapeutic regimens. One reason for this is the pleiotropic nature of many cytokines. For example, interleukin (IL)-11 is a functionally pleiotropic molecule (1,2), initially

characterized by its ability to stimulate proliferation of the IL-6-dependent plasmacytoma cell line, T11 65 (3). Other biological actions of IL-11 include induction of multipotential haemopoietin progenitor cell proliferation (4,5,6), enhancement of megakaryocyte and platelet formation (7,8,9,10), stimulation of acute phase protein synthesis (11) and inhibition of adipocyte lipoprotein lipase activity (12, 13).

Interleukin-13 (IL-13) is another important cytokine which shares a number of structural characteristics with interleukin-4 (IL-4) [reviewed in 14 and 15]. The genes for IL-4 and IL-13 have a related intron/exon structure and are located close together on 10 chromosome 5 in the human and the syntonic region of chromosome 11 in the mouse (14, 15). At the protein level, IL-4 and IL-13 share approximately 30% amino acid identity, including four cysteine residues. Biologically, IL-13 and IL-4 are also similar, being produced by activated T-cells and acting upon macrophages to induce differentiation and suppress the production of inflammatory cytokines. Additionally, 15 human IL-13 may act as a co-stimulatory signal for B-cell proliferation and affect immunoglobulin isotype switching (14, 15). The diverse and pleiotropic function of IL-13 and other haemopoietic cytokine makes this molecule an important group to study, especially at the level of interaction of the cytokine with its receptors. Manipulation and control of cytokine receptors and of cytokine-receptor interaction is potentially very 20 important in many therapeutic situations, especially where the target cytokine is functionally pleiotropic and it is desired to block certain functions of a target cytokine but not all functions.

Research into IL-13 and its receptor has been hampered due to the inability to clone 25 genetic sequences encoding all or part of the IL-13 receptor. In accordance with the present invention, genetic sequences have now been cloned encoding the IL-13 receptor  $\alpha$ -chain. The availability of these genetic sequences permits the development of a range of therapeutic and diagnostic agents capable of modulating IL-13 activity as well as the activity of cytokines related at the level of IL-13 receptor structure.

Accordingly, one aspect of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an haemopoietin receptor  $\alpha$ -chain from an animal or a component, fragment, 5 part, derivative, homologue or analogue thereof.

More particularly, the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding the IL-13 receptor  $\alpha$ -chain from an animal or a component, fragment, part, derivative, homologue 10 or analogue thereof.

In a related embodiment, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding the IL-4 receptor  $\alpha$ -chain from an animal or a component, fragment, part, 15 derivative, homologue or analogue thereof.

Preferably, the animal is a mammal or a species of bird. Particularly, preferred mammals include humans, laboratory test animals (e.g. mice, rabbits, guinea pigs), livestock animals (e.g. sheep, horse, pigs, cows), companion animals (e.g. dogs, cats) or 20 captive wild animals (e.g. kangaroos). Although the present invention is exemplified with respect to mice and humans, the scope of the subject invention extends to all animals and birds.

The present invention is predicated in part on an ability to identify members of the 25 haemopoietin receptor family on the basis of sequence similarity. Based on this approach, a genetic sequence was identified in accordance with the present invention which encodes the IL-13  $\alpha$ -chain. The expressed genetic sequence is referred to herein as "NR4". NR4 has an apparent molecular weight when synthesised by transfected COS cells of from about 50,000 to about 70,000 daltons, and more preferably from about 30 55,000 to about 65,000 daltons. NR4 binds to IL-13 with low affinity and is considered, therefore, to be IL-13 receptor  $\alpha$ -chain. Accordingly, the terms "NR4" and "IL-13" receptor  $\alpha$ -chain" (or "IL-13  $\alpha$ ") are used interchangeably throughout the subject

specification. Furthermore, in accordance with the present invention, IL-13 binding to its receptor has been found to be competitively inhibited by IL-4 or a component thereof which may provide a method for controlling IL-13-receptor interaction and which may also provide a basis for the preparation and construction of mimetics.

5

Another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding IL-13 receptor  $\alpha$ -chain having an amino acid sequence as set forth in SEQ ID NO:2 or having at least about 50% similarity to all or part thereof. Preferably, the percentage similarity is at least about 60%, more preferably at 10 least about 70%, even more preferably at least about 80-85% and still even more preferably at least about 90-95% or greater.

A further embodiment of the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides encoding the IL-13 receptor  $\alpha$ -chain and having 15 a nucleotide sequence substantially as set forth in SEQ ID NO:1 or having at least about 50% similarity to all or part thereof. Preferably, the percentage similarity is at least about 60%, more preferably at least about 70%, even more preferably at least about 80-85% and still even more preferably at least about 90-95% or greater.

20 Still another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding IL-13 receptor  $\alpha$ -chain having an amino acid sequence as set forth in SEQ ID NO:4 or having at least about 50% similarity to all or part thereof. Preferably, the percentage similarity is at least about 60%, more preferably at least about 70%, even more preferably at least about 80-85% and still even more 25 preferably at least about 90-95% or greater.

Yet still a further embodiment of the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides encoding the IL-13 receptor  $\alpha$ -chain and having a nucleotide sequence substantially as set forth in SEQ ID NO:3 or having at 30 least about 50% similarity to all or part thereof. Preferably, the percentage similarity is at least about 60%, more preferably at least about 70%, even more preferably at least about 80-85% and still even more preferably at least about 90-95% or greater.

Accordingly, the present invention extends to the sequence of nucleotides set forth in SEQ ID NO:1 or 3 or the sequence of amino acids set forth in SEQ ID NO:2 or 4 or single or multiple nucleotide or amino acid substitutions, deletions and/or additions thereto.

5

The present invention further extends to nucleic acid molecules capable of hybridising under low stringency conditions to the nucleotide sequence set forth in SEQ ID NO:1 or 3 or a complementary form thereof.

- 10 For the purposes of defining the level of stringency, reference can conveniently be made to Maniatis *et al* (1982) at pages 387-389 which are incorporated herein by reference where the washing step at paragraph 11 is considered herein to be high stringency. A low stringency wash is defined herein to be 0.1-0.2xSSC, 0.1% w/v SDS at 55-65°C for 20 minutes and a medium level of stringency is considered herein to be 2xSSC,
- 15 0.1% w/v SSC at  $\geq 45^{\circ}\text{C}$  for 20 minutes. The alternative conditions are applicable depending on concentration, purity and source of nucleic acid molecules.

Yet another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a sequence which

- 20 encodes an IL-13 receptor  $\alpha$ -chain, said nucleic acid molecule having a nucleotide sequence substantially as set forth in SEQ ID NO:1 or 3 or a nucleic acid molecule which encodes a structurally similar IL-13 receptor  $\alpha$ -chain or a derivative thereof and which is capable of hybridising to the nucleotide sequence substantially as set forth in SEQ ID NO:1 or 3 or a complementary form thereof under low stringency conditions.

25

Still yet another aspect of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a sequence which encodes the IL-13 receptor  $\alpha$ -chain having an amino acid sequence substantially as set forth in SEQ ID NO:2 or 4 or comprises a nucleotide sequence coding for an

- 30 amino acid sequence having at least about 50% similarity to the sequence set forth in SEQ ID NO:2 or 4 and is capable of hybridising to the sequence set forth in SEQ ID NO:1 or 3 under low stringency conditions.

The nucleic acid molecules contemplated by the present invention are generally in isolated form and are preferably cDNA or genomic DNA molecules. In a particularly preferred embodiment, the nucleic acid molecules are in vectors and most preferably expression vectors to enable expression in a suitable host cell. Particularly useful host

5 cells include prokaryotic cells, mammalian cells, yeast cells and insect cells. The cells may also be in the form of a cell line.

According to this aspect of the present invention there is provided an expression vector comprising a nucleic acid molecule encoding the IL-13 receptor  $\alpha$ -chain as hereinbefore

10 described, said expression vector capable of expression in a particularly host cell.

Another aspect of the present invention contemplates a recombinant polypeptide comprising a sequence of amino acids substantially as set forth in SEQ ID NO:2 or 4 or having at least about 50% similarity to all or part thereof. Preferably, the percentage

15 similarity is at least about 60%, more preferably at least about 70%, even more preferably at least about 80-85% and still even more preferably at least about 90-95% or greater.

The recombinant polypeptide contemplated by the present invention includes, therefore,

20 components, parts, fragments, derivatives, homologues or analogues of the IL-13 receptor  $\alpha$ -chain and is preferably encoded by a nucleotide sequence substantially set forth in SEQ ID NO:1 or 3 or a molecule having at least about 50% similarity to all or part thereof or a molecule capable of hybridising to the nucleotide sequence set forth in SEQ ID NO:1 or 3 or a complementary form thereof. The recombinant molecule may

25 be glycosylated or non-glycosylated. When in glycosylated form, the glycosylation may be substantially the same as naturally occurring IL-13 receptor  $\alpha$ -chain or may be a modified form of glycosylation. Altered or differential glycosylation states may or may not affect binding activity of the IL-13 receptor  $\alpha$ -chain.

30 The recombinant IL-13 receptor  $\alpha$ -chain may be in soluble form or may be expressed on a cell surface or conjugated or fused to a solid support or another molecule.

The present invention extends to chemical analogues of the recombinant IL-13 receptor  $\alpha$ -chain.

Chemical analogues of the recombinant IL-13 receptor  $\alpha$ -chain contemplated herein

5 include, but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide synthesis and the use of crosslinkers and other methods which impose conformational constraints on the peptides or their analogues.

10 Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with  $\text{NaBH}_4$ ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6, trinitrobenzene sulphonic acid (TNBS);

15 acylation of amino groups with succinic anhydride and tetrahydronaphthalic anhydride; and pyridoxylation of lysine with pyridoxal-5'-phosphate followed by reduction with  $\text{NaBH}_4$ .

20 The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

25 The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

30 Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetrinitromethane to form a 3-nitrotyrosine derivative.

5

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

10 Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids.

15

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having  $(CH_2)_n$  spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-

20 hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of  $C_\alpha$  and  $N_\alpha$ -methylamino acids, introduction of double bonds between  $C_\alpha$  and  $C_\beta$  atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming  
25 an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

30 Chemical modification of the recombinant IL-13 receptor  $\alpha$ -chain may be important, for example, to increase serum half-life, to protect the molecule from enzymatic degradation and/or for diagnostic purposes.

The recombinant IL-13 receptor  $\alpha$ -chain contemplated by the present invention is useful in the development of a range of agonists and antagonists of IL-13-receptor interaction. The recombinant molecule may also be used in the development of diagnostic agents.

5

Particularly useful agents encompassed by this aspect of the present invention are antibodies to the recombinant IL-13 receptor  $\alpha$ -chain. The antibodies may be monoclonal or polyclonal and are particularly useful as antagonists of IL-13-receptor binding or as diagnostic agents to qualitate or quantitate the presence of the IL-13  
10 receptor  $\alpha$ -chain. These antibodies may also be useful in the screening of similar components in other receptors such as IL-4 receptors.

15 Other agonists and antagonists include chemical molecules which, for example, structurally, functionally or electrochemically mimic or have similarities to IL-13 receptor  $\alpha$ -chain or which comprise a solubilised form of the IL-13 receptor  $\alpha$ -chain.

Such agents are useful in modulating IL-13-receptor interaction and these are useful in enhancing or diminishing IL-13 related activities. This may be particularly important for cancers or tumours involving or resulting from excess IL-13 or from aberrant IL-13  
20 molecules or to promote IL-13 function in the treatment of a range of conditions such as, but not limited to, immune deficiency.

The present invention further contemplates ribozyme and antisense molecules useful in reducing IL-13 receptor  $\alpha$ -chain expression.

25

The present invention encompasses, therefore, pharmaceutical and diagnostic compositions comprising recombinant IL-13 receptor  $\alpha$ -chain or parts thereof, antibodies thereto, agonists or antagonists thereof or genetic molecules such as ribozymes, antisense molecules and constructs useful in co-suppression.

30

The present invention is further described by the following non-limiting Figures and Examples.

5 In the Figures:

**Figure 1** is a representation of the nucleotide [SEQ ID NO:1] and predicted amino acid [SEQ ID NO:2] sequence of murine NR4. The untranslated region is shown in lower case and the translated region in upper case. The conventional one-letter code for amino acids is employed, potential asparagine linked glycosylation sites are underlined and the conserved cysteine residues and WSXWS motif of haemopoietin receptor family members are shown in bold. The predicted signal sequence is underlined in bold while the transmembrane domain is underlined with dashes. The sequence shown is a composite derived from the analysis of 8 cDNA clones derived from 3 libraries. The 5'-end of the sequence (nucleotides -60 to 351) is derived from a single cDNA clone but is also present in genomic DNA clones that have been isolated.

**Figure 2** is a photographic representation showing northern analysis of murine NR4 mRNA expression in selected tissues and organs.

20 **Figure 3** is a graphical representation depicting saturation isotherms of  $^{125}\text{I}$ -IL-13 and  $^{125}\text{I}$ -IL-4 binding; saturation isotherms depicted as Scatchard plots of IL-4 (○) and IL-13 (●) binding to (A) COS cells expressing the IL-13R $\alpha$  (NR4), (B) CTLL cells and (C) CTLL cells expressing the IL-13R $\alpha$  (NR4). Data have been normalised to  $1 \times 10^4$  COS cells and  $1 \times 10^6$  CTLL cells and binding was carried out on ice for 2 to 4 hours.

25 **Figure 4** is a graphical representation showing specificity of IL-4 and IL-13 binding; the ability of IL-4 (○) and IL-13 (●) to compete for  $^{125}\text{I}$ - $^{125}\text{I}$ -IL-13 binding to (A) COS cells expressing the IL-13R $\alpha$  (NR4) and (C) CTLL cells expressing the IL-13R $\alpha$  (NR4) or to compete for IL-4 binding to (B) CTLL cells and (D). CTLL cells expressing the IL-13R $\alpha$  (NR4) binding was carried out on ice for 2 to 4 hours and the data have been expressed as a percentage of the specific binding observed in the absence of a competitor

(■).

**Figure 5** is a graphical representation showing factor dependent proliferation of cells expressing NR4. Two hundred (A) CTLL cells or (B) CTLL cells expressing the IL-5 13R $\alpha$  (NR4) were incubated in the absence of cytokine (■) or with various concentrations of IL-2 (□), IL-4 (○) or IL-13 (●). After 48 hours viable cells were counted and data was expressed as a percentage of the number of viable cells observed with a maximal concentration of IL-2.

10 **Figure 6** is a photographic representation showing cross-species conservation of NR4 (IL-13R $\alpha$ ) gene.

15 **Figure 7** is a representation of the nucleotide and corresponding amino acid sequence of murine and human NR4 (IL-13R $\alpha$ ) genes. The nucleotide and predicted amino acid sequence of human (H) and murine (M) IL-13R $\alpha$  (NR4) were aligned by eye, with gaps (-) inserted to optimise the alignment. The numbering is for the murine clone, nucleotides that form part of the coding region are shown in upper case, whilst those of the untranslated regions are shown in lower case. Amino acids identical between the predicted murine and human proteins are indicated by (\*). DNA encoding the murine 20 signal sequence is underlined, with A26 or T27 being the predicted first amino acid of the mature protein.

**Figure 8** is a photographic representation showing NR4 expression in mouse tissues.

The following single and three letter abbreviations for amino acid residues are used in the specification:

5

	Amino Acid	Three-letter Abbreviation	One-letter Symbol
	Alanine	Ala	A
10	Arginine	Arg	R
	Asparagine	Asn	N
	Aspartic acid	Asp	D
	Cysteine	Cys	C
	Glutamine	Gln	Q
15	Glutamic acid	Glu	E
	Glycine	Gly	G
	Histidine	His	H
	Isoleucine	Ile	I
	Leucine	Leu	L
20	Lysine	Lys	K
	Methionine	Met	M
	Phenylalanine	Phe	F
	Proline	Pro	P
	Serine	Ser	S
25	Threonine	Thr	T
	Tryptophan	Trp	W
	Tyrosine	Tyr	Y
	Valine	Val	V
	Any residue	Xaa	X
30			

### EXAMPLE 1

#### Isolation of genomic and cDNAs encoding NR4

*ApoI* digested genomic DNA, extracted from an embryonal stem cell line, was cloned 5 into the  $\lambda$ ZAPII bacteriophage (Stratagene, LaJolla, CA). Approximately  $10^6$  plaques from this library were screened with a  $^{32}$ P-labelled oligonucleotide corresponding to the sequence Trp-Ser-Asp-Trp-Ser [SEQ ID NO:3] (16). Positively hybridising clones were sequenced using an automated DNA sequencer according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). One clone appeared to encode for 10 part of a new member of the haemopoietin receptor family. Oligonucleotides were designed on the basis of this genomic DNA sequence and were used in the conventional manner to isolate clones from mouse peritoneal macrophage (Clontech Laboratories, Palo Alto, CA), mouse skin, mouse lung, mouse kidney, and WEHI-3B (Stratagene, LaJolla, CA)  $\lambda$ -bacteriophage cDNA libraries.

15

### EXAMPLE 2

#### Construction of expression vectors and transfection of cells

Using PCR, a derivative of the NR4 cDNA was generated which encoded for the IL-3 signal sequence and an N-terminal FLAG epitope-tag preceding the mature coding 20 region of NR4 (Thr27 to Pro424; Figure 1). The PCR product was cloned into the mammalian expression vector pEF-BOS (17). Constructs were sequenced in their entirety prior to use. Cells were transfected and selected as previously described (16, 18).

25

### EXAMPLE 3

#### Northern blots

Northern blots were performed as previously described (16). The source of hybridisation probes was as follows: NR4 - a PCR product from nucleotide 32 to 984 (Figure 1) and GAPDH - a cDNA fragment spanning nucleotides (19) [REF REQUIRED].

30

#### EXAMPLE 4

##### Cytokines and binding experiments using radioiodinated cytokines

IL-2, IL-4, IL-7, IL-9, IL-13 and IL-15 were obtained commercially (R & D Systems, Minneapolis MN). For radioiodination, cytokines were dissolved at a concentration of 100 µg/ml in 10 mM sodium phosphate, 150 mM NaCl (PBS), 0.02% v/v Tween 20 and 0.02% w/v sodium azide at pH 7.4. An amount of 2µg of IL-13 was radioiodinated using the iodine monochloride method (20, 21), while 2µg of IL-4 was radiolabelled using dioiodo-Bolton-Hunter reagent (16). Binding studies and determination of the specific radioactivity and bindability of labelled cytokines were performed as previously described (2).

#### EXAMPLE 5

##### Proliferation Assays

The proliferation of Ba/F3 and CTLL cells in response to cytokines was measured in Lux 60 microwell HL-A plates (Nunc Inc. IL, USA). Cells were washed three times in DME containing 20% v/v new born calf serum and resuspended at a concentration of  $2 \times 10^4$  cells per ml in the same medium. Aliquots of 10µl of the cell suspension were placed in the culture wells with 5µl of various concentrations of purified recombinant cytokines. After 2 days of incubation at 37°C in a fully humidified incubator containing 10% v/v CO<sub>2</sub> in air, viable cells were counted using an inverted microscope.

#### EXAMPLE 6

##### Cloning and Characterisation of Murine NR4

A library was constructed in λZAPII using *ApoI* digested genomic DNA from embryonal stem cells and screened with a pool of <sup>32</sup>P-labelled oligonucleotides encoding the amino acid sequence Trp-Ser-Asp-Trp-Ser [SEQ ID NO:3] found in many members of the haemopoietin receptor family. One hybridising bacteriophage was found to contain a genomic clone that appeared to encode part of a novel member of the haemopoietin receptor family. This receptor was given the operational name NR4. The sequence of the genomic clone was used to isolate cDNAs encoding NR4 from WEHI-3B cell, peritoneal macrophage, bone marrow, skin and kidney libraries. A composite of the

nucleotide sequence [SEQ ID NO:1] and predicted amino acid sequence [SEQ ID NO:2] of these cDNAs is shown in Figure 1. The NR4 cDNA is predicted to encode for a protein of 424 amino acid residues, containing a putative signal sequence and transmembrane domain. The extracellular region of the protein containing a putative 5 signal sequence and transmembrane domain. The extracellular region of the protein contained an immunoglobulin-like domain (amino acids 27-117), in addition to a typical haemopoietin receptor domain (amino acids 118-340) which includes four conserved cysteine residues and the characteristic Trp-Ser-Asp-Trp-Ser [SEQ ID NO:3] motif (Figure 1). The cytoplasmic tail of the new receptor was 60 amino acids in length.

10

#### **EXAMPLE 7**

##### **Expression pattern of NR4 cDNA**

The pattern of NR4 mRNA expression was examined by Northern analyses. Two hybridising species of 5.2 and 2.2 kb in length were detected in mRNA from most 15 tissues (Figure 2). NR4 mRNA was not detectable in skeletal muscle (Figure 2). Figure 8 shows expression of NR4 in mouse tissues.

#### **EXAMPLE 8**

##### **NR4 encodes the IL-13 receptor $\alpha$ -chain (IL-13R $\alpha$ ) - a specific 20 binding subunit of the IL-13 receptor**

The apparent molecular weight is from about 50,000 to about 70,000 daltons and more particularly about 55,000 to about 65,000 daltons for NR4 expressed in COS cells estimated from Western blots using an anti-FLAG antibody, suggested that NR4 might encode the binding subunit of the IL-13 receptor. In order to test this possibility NR4 25 was expressed in COS cells. Untransfected COS cells expressed relatively low levels of IL-4 and IL-13 receptors. Upon transfection with a plasmid containing the NR4 cDNA, the number of IL-13 receptors but not IL-4 receptors expressed by COS cells was dramatically increased (Figure 3A; 100,000 to 500,000 receptors per cell). The affinity of IL-13 for NR4 expressed by COS cells was low ( $K_D$ ~2-10 nM) and binding 30 was specific since it was in competition with unlabelled IL-13 but not other cytokines including IL-2, IL-4, IL-7, IL-9 or IL-15 (Figure 4A). These results suggest that NR4 is the IL-13 receptor  $\alpha$ -chain (IL-13R $\alpha$ ).

### EXAMPLE 9

#### The IL-13R $\alpha$ (NR4) and the IL-4R $\alpha$ are shared components of the IL-4 and IL-3 receptors

In order to investigate the relationship between IL-4 and IL-13 receptors, the IL-4 responsive cell line CTLL was examined. Parental CTLL cells expressed a single class of IL-4 receptor ( $K_D \sim 660$  pM;  $\sim 3600$  receptors per cell) but no detectable IL-13 receptors (Figure 3B). The IL-4 receptors expressed by CTLL cells appeared to be specific since binding of  $^{125}\text{I}$ -IL-4 was in competition with unlabelled IL-4 but not IL-13 (Figure 4B). Upon expression of the IL-13R $\alpha$  (NR4) in CTLL cells no change was observed in the number or affinity of IL-4 receptors, while a single class of high affinity IL-13 receptors was detected (Figure 3C;  $K_D \sim 75$  pM; 1350 receptors per cell). The affinity of IL-13 for the IL-13R $\alpha$  (NR4) expressed in CTLL cells was higher than in COS cells, suggesting that the former expressed a protein capable of interacting with the IL-13R $\alpha$  (NR4) to increase the affinity for IL-13. A likely candidate based on previous studies is the IL-4R $\alpha$ . In order to explore this possibility the ability of IL-4 to compete with  $^{125}\text{I}$ -IL-13 for binding to CTLL cells expressing the IL-13R $\alpha$  (NR4) was assessed. Figure 4B shows that IL-4 and IL-13 were equally effective in competing for  $^{125}\text{I}$ -IL-13 binding ( $\text{IC}_{50} \sim 300$  pM; Figure 4C) and, in addition, were able to compete with  $^{125}\text{I}$ -IL-4 for binding ( $\text{IC}_{50} \sim 300$  pm; Figure 4D).

20

### EXAMPLE 10

#### Expression of the IL-13R $\alpha$ (NR4) is necessary for transduction of a proliferative signal by IL-13

CTLL cells require the addition of exogenous cytokines for survival and proliferation. IL-2 was found to be a potent proliferative stimulus for CTLL cells ( $\text{EC}_{50} \sim 100$ -200 pM), while IL-4 was relatively weak ( $\text{EC}_{50} 2$ -7 nM) and IL-13 was inactive (Figure 5A). Expression of the IL-13R $\alpha$  (NR4) in CTLL cells resulted in the ability to survive and proliferate weakly in response to IL-13 ( $\text{EC}_{50} \sim 700$  pM) and to proliferate somewhat more strongly than parental cells in response to IL-4 ( $\text{EC}_{50} \sim 700$  pM; Figure 5B).

## EXAMPLE 11

### Cloning of Human IL-13R $\alpha$ (NR4)

In order to determine whether genes homologous to murine IL-13R $\alpha$  (NR4) exist in other vertebrate species, a probe encompassing nucleotides 840 to 1270 of murine IL-13R $\alpha$  (NR4) was hybridised to *Eco*RI digested genomic DNA from various species. Hybridisation was carried out in 500 mM Na<sub>2</sub>HPO<sub>4</sub> (~5xSSC) at 50°C overnight. The filter was washed in 40 mM Na<sub>2</sub>HPO<sub>4</sub> (~0.2xSSC) at 50°C for 2 hours and exposed to autoradiographic film for 48 hours. Figure 6 illustrates that relatively few (1 to 5) hybridising bands are observed in genomic DNA from various species, including human. This suggests that it is feasible to clone human IL-13R $\alpha$  (NR4) using a murine cDNA probe. A human bone marrow cDNA library clones in the  $\lambda$ ZAPII bacteriophage was therefore screened with two probes (nucleotides 82-840 and 840 to 1270) from the murine IL-13R $\alpha$  (NR4) cDNA. Hybridisation was carried out overnight in 6xSSC, 0.1% w/v SDS at 42°C. Filters were washed at 2xSSC, 0.1% w/v SDS at 50°C for 2 hours and exposed for 48 hours to autoradiographic film. Plaques that hybridised to both murine IL-13R $\alpha$  (NR4) probes were picked and purified in the conventional manner. The cDNA inserts from the hybridising bacteriophage were excised into the pBluescript plasmid and sequenced in their entirety using an ABI automated sequencer. Figure 7 shows a composite of the sequence of the clones isolated and reveals that the clones encode a protein that shares a high degree of sequence similarity with murine IL-13R $\alpha$  (NR4). The clones encode for the entire mature coding region of the protein, but lack the initiation methionine and the signal sequence; the high degree of sequence similarity (311/401 amino acids ~ 78%) predicates that this cDNA is the human homologue of the murine IL-13R $\alpha$  (NR4).

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH

(ii) TITLE OF INVENTION: A NOVEL HAEMOPOIETIN RECEPTOR AND GENETIC SEQUENCES ENCODING SAME - II

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: DAVIES COLLISON CAVE  
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(C) CITY: MELBOURNE  
(D) STATE: VICTORIA  
(E) COUNTRY: AUSTRALIA  
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: AU PROVISIONAL  
(B) FILING DATE: 23-OCT-1995

(viii) ATTORNEY/AGENT INFORMATION:

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(ix) TELECOMMUNICATION INFORMATION:

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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1680 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 1..1272
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 424 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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290 295 300

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1248 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..1203

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 401 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Ser Ser Asn Cys Ser Leu Trp Tyr Phe Ser His Phe Gly Asp Lys Gln  
35 40 45

Asp Lys Lys Ile Ala Pro Glu Thr Arg Arg Ser Ile Glu Val Pro Leu  
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Asn Glu Arg Ile Cys Leu Gln Val Gly Ser Gln Cys Ser Thr Asn Glu  
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Gly Asp Pro Glu Ser Ala Val Thr Glu Leu Gln Cys Ile Trp His Asn  
100 105 110

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385 390 395 400

Gln

DATED this 22nd day of December, 1995

THE WALTER AND ELIZA HALL INSTITUTE  
OF MEDICAL RESEARCH  
By Its Patent Attorneys  
DAVIES COLLISON CAVE

1 M A R S A S L G E L I Y C L L W T A T V  
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 161 A C T C T C A G A T A T T T T A G T C A C T T T G A C C A C A C G G A T A A G A A A A T T G C T C C A C A A A C T  
 62 T I R Y P S H F D D Q Q D K R I A P E T  
 241 C A T C T A A A C A C G G A T T A C C C T G G A T G A G A A A A T C T G T C T G C A C C G T G G C T C A G T G T  
 81 H R K E S L P L O E K I C L Q V G S Q C  
 301 A G T G C C A A T G A A A G T G A G A A G C C T A G G C C T T T G G T G A A A A A G T G C A T C T C A C C C C T C O A A  
 101 S A N E S E K P S P L V K X C I S P F E  
 361 O C T G A T C C T G A G T C C G C T G T G R C T G A G G T C A A G T G C A T T T G C A T A A C T G A G G T A T A T G  
 121 G D P E S A V T E L K C I W H N L 3 Y M  
 421 A A G T T T C C T G G C T C C C T G G A A G G A T A C A A C C C T G A C A C A C T A T A C T C T G T A C T A T  
 141 K C S W L P G R N T S P D T H Y T L Y Y  
 481 T C C T A C C A C C G C T G A G A A A A G T G C T C A T G T G A A A C A C T C T A T A G A G A A G G T C A A C  
 161 W Y T S S L E K S R Q C E N I Y R E G Q H  
 541 A T T C C T T O T C C T T T A A T T G A C T A A A G T G G A A C C T A C T T T G C A A C T C A G A A C G T C A A  
 181 I A C S F K L T K V E P S F B K Q N V Q  
 501 A T A A T G T C A A G G A T A A T G T G G G A A A A T T A G G C C A T C T G C A A A A T A C T C T C T T A C T  
 201 I M V K D N A Q X I R P S C K I V S L T  
 561 T C C T A T G T G A A A C C T G A T C C T C A C A T A T T A A C A T T T C T C T C A A A A A T O C T C C T T A  
 231 S Y V K P D P P H I S K H L L L K N G A L  
 721 T T A G T G C A G T G G A A G A A T C C A C A A A T T T T A G A A G G C A G T G C T T A A C T T A T G A A G T G G A G  
 241 L V Q W X K N P Q N F R S R C L T Y E V E  
 781 G T C A A T A A T A C T C A A A C C C A C C O A C A T A T A T T T A G A G G C T T G A A G C C G C A C A A N T C C A G  
 261 V N N T Q T D R H N I L E V E S D K C Q  
 841 A A T T C C G A A T C T G A T A G A A A C A T G G A G G C T A C A M T T G T T C C A A C T C C T G G T G T C T T  
 281 N S E S D R N M B G T S C F Q L P G V L  
 901 G C C G A C O C T G T C T A C A C A G T C A G A G T R A G A G T C A A A A C A A C A A G T T A O C T T T G A T G A C  
 301 A D A V Y T V R V R V K T N K L C F D D  
 961 A A C A A A C T G T G G A G T C A T T G A C T G A G G C A C A G A G T A A G G T A A G G G C A A A A C T C C A C C  
 321 N K L W S D W G E A O S T G K E O N E T  
 1021 T T T A C A C C A C C A T G T A C T C A C C A T T C C A G C T C T C C A G C T C A G T C A T A A T C C C  
 341 F Y T T M L L T I P U F V A V A V I I L  
 1081 C T T T P T A C C I G A A A A G C T T A A C A T C A T T A T T T C T C C A A M T T C C T G A T C C T G C C A A G  
 361 L F Y L K R L K I I I F P P P I P D P G K  
 1141 A T T T T T A A G G A A A T G T T G G A G A C C C A G A A T G A T C A T C C C T G C A C T G G R G A A G T A T G A C  
 381 I F K E M F G D Q N D D T L H W K K Y D  
 1201 A T C T A T G A G A A A C A A T C C A A A G A G A A A N C G G A T T C T G T A G T G C T C A T A G A A A A C C T G A A G  
 401 I Y E K Q S K E E T D S V V L I E N L K  
 1261 A A A C C A G C T C C T T C A t g g g g a a g t g a t t c t t t t g e c t t c a a t g t g a c c c t g t g a a  
 421 K A A P \*

**FIGURE 1**

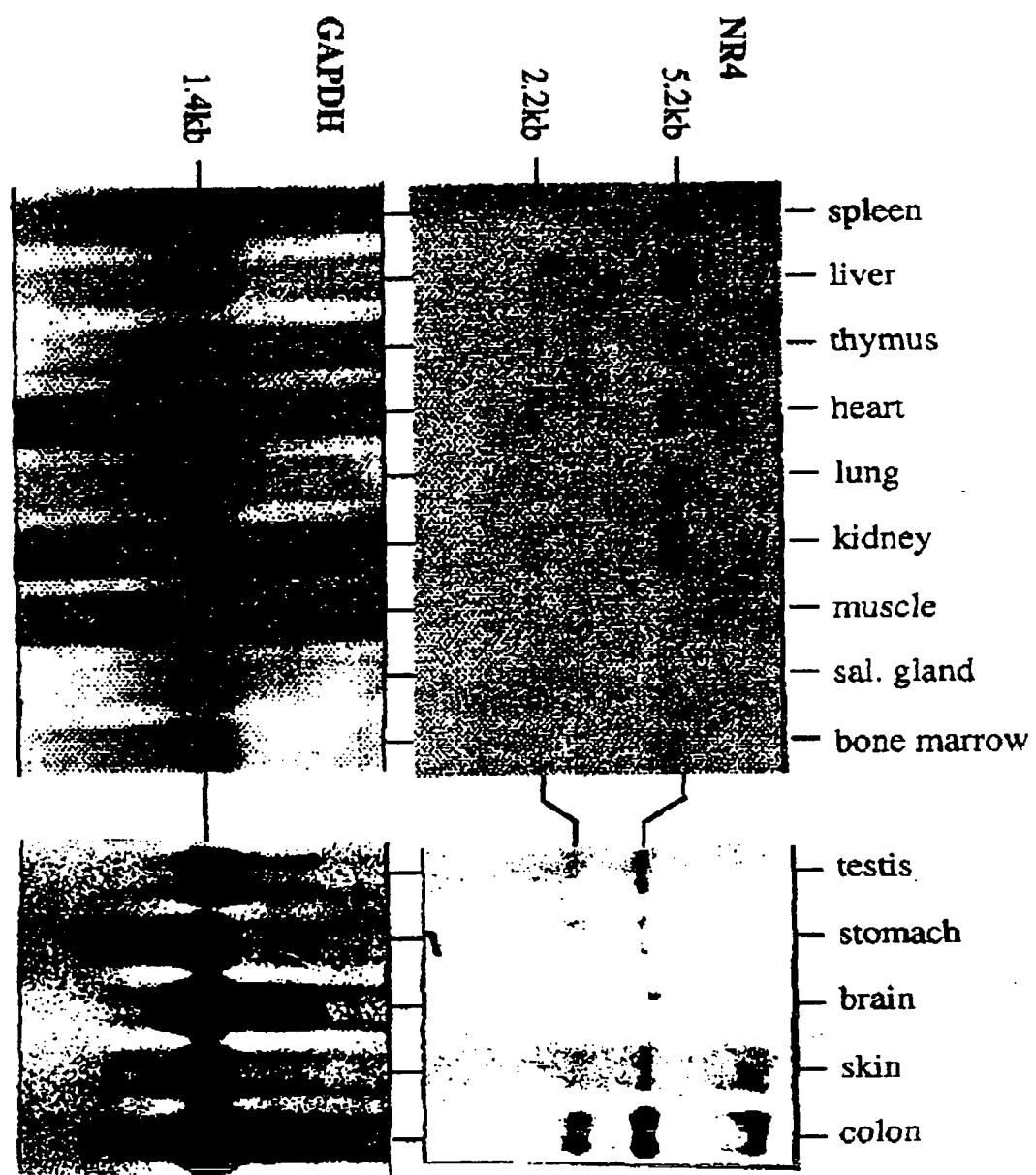


FIGURE 2

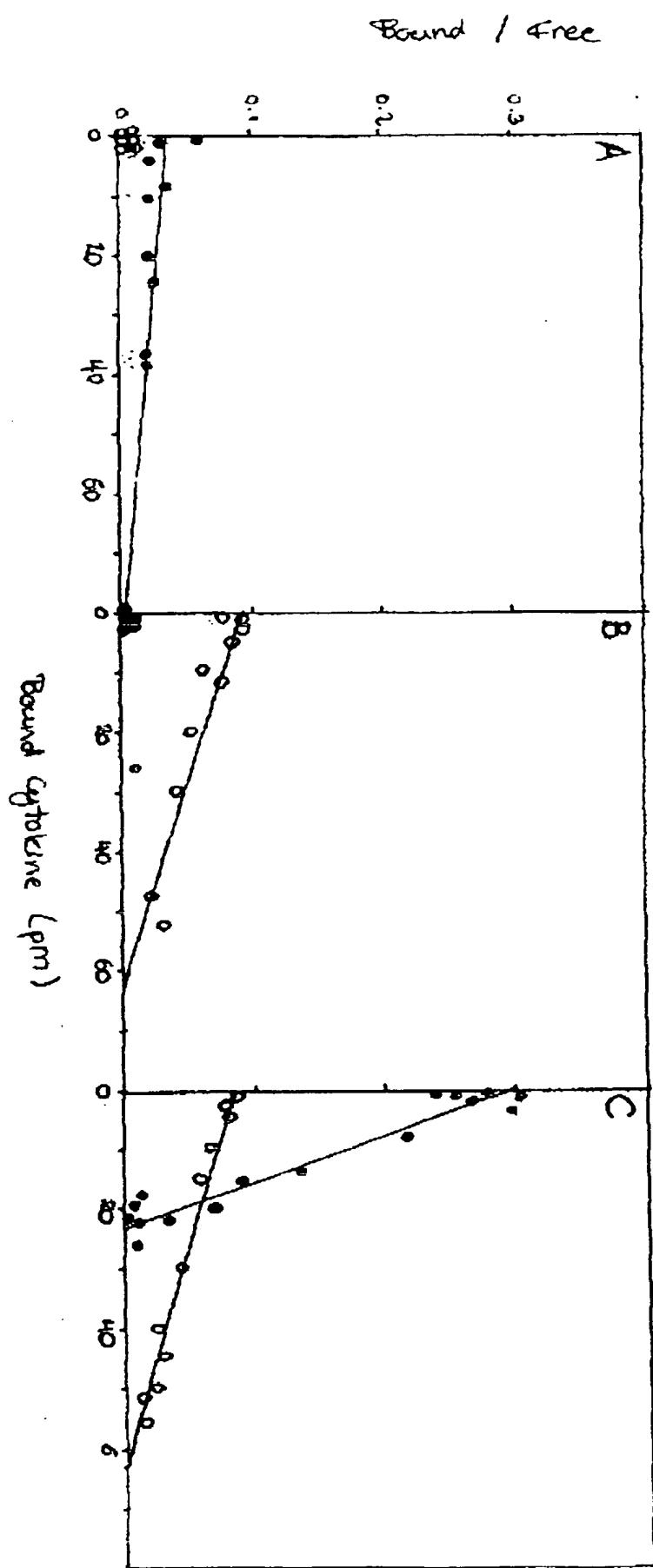


FIGURE 3

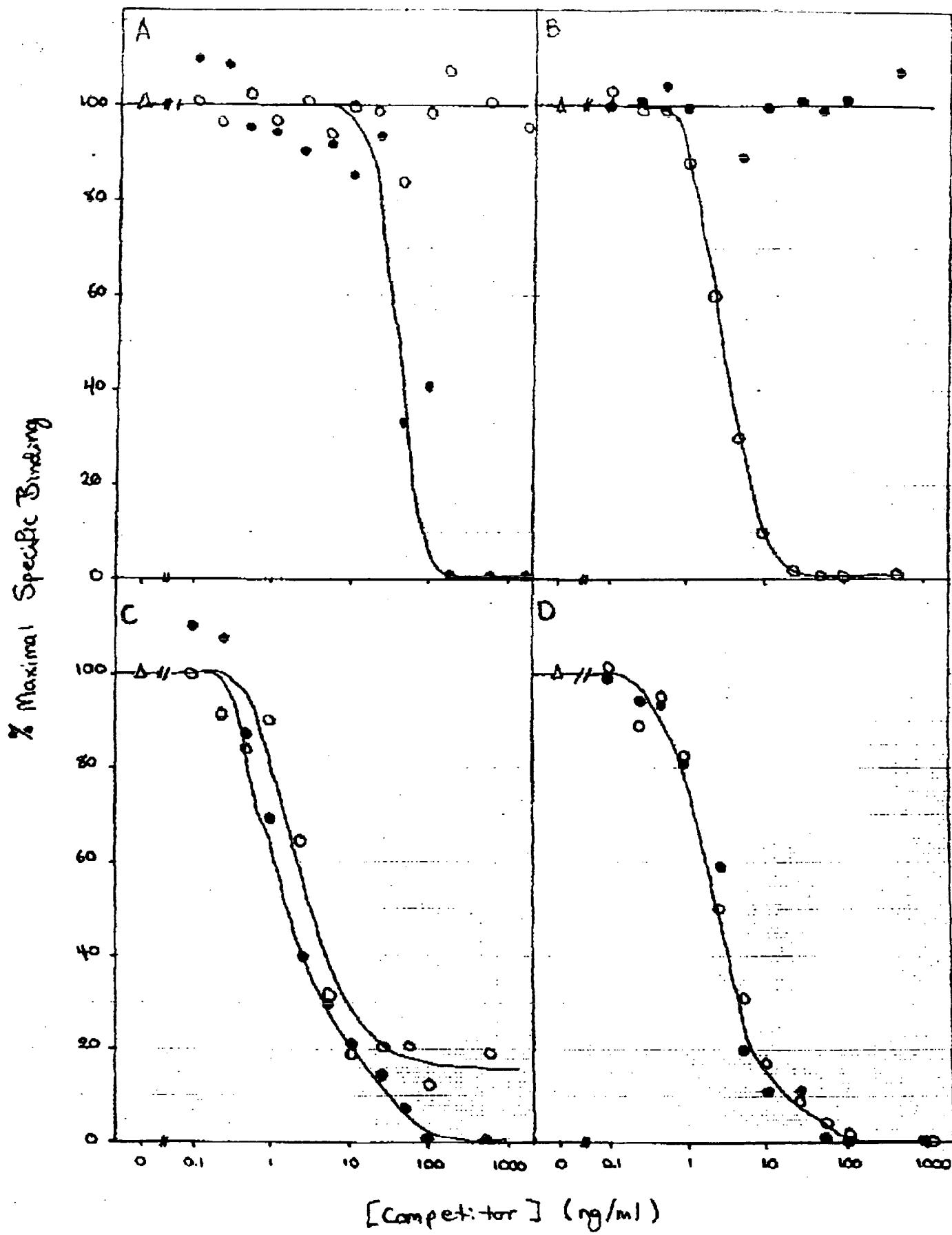


FIGURE 4

% Maximum Number of Viable Cells.

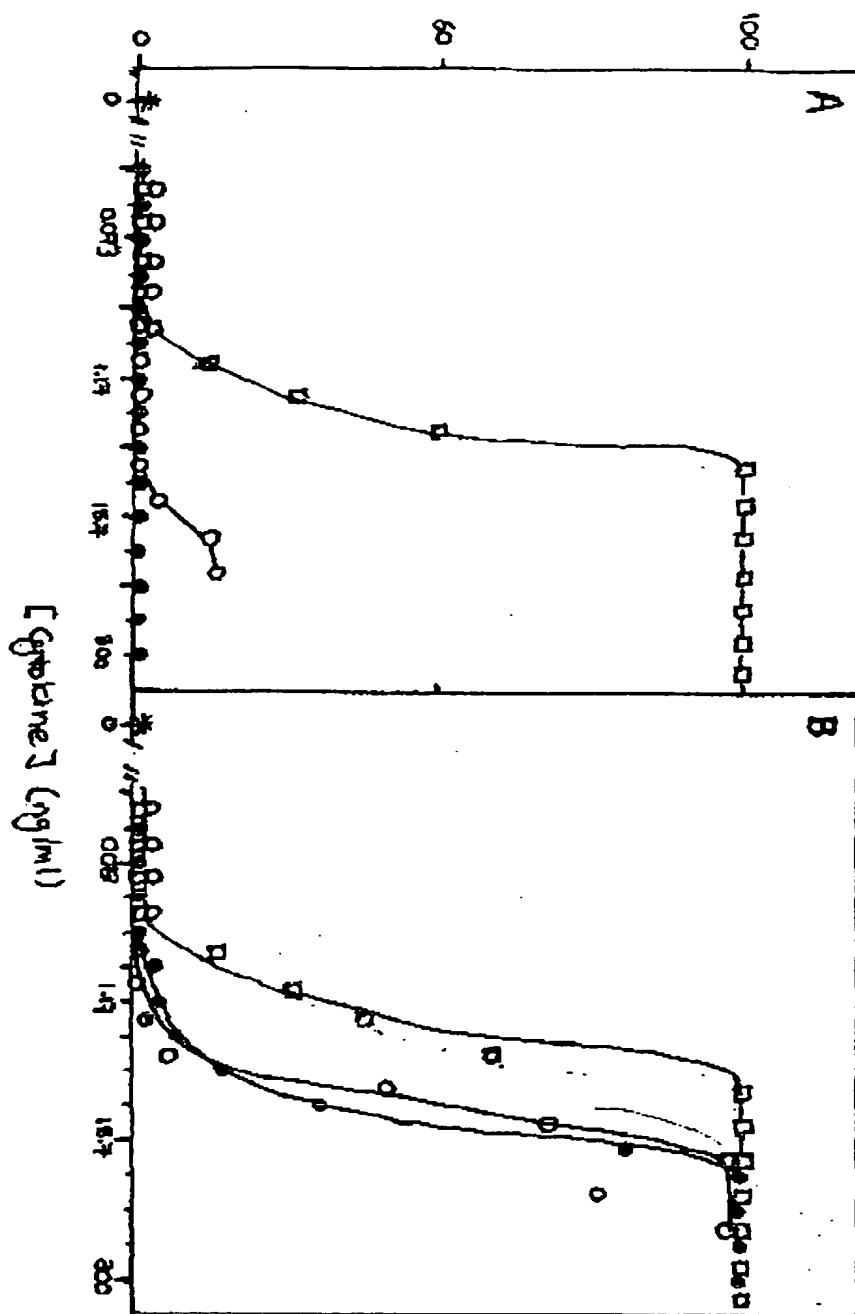
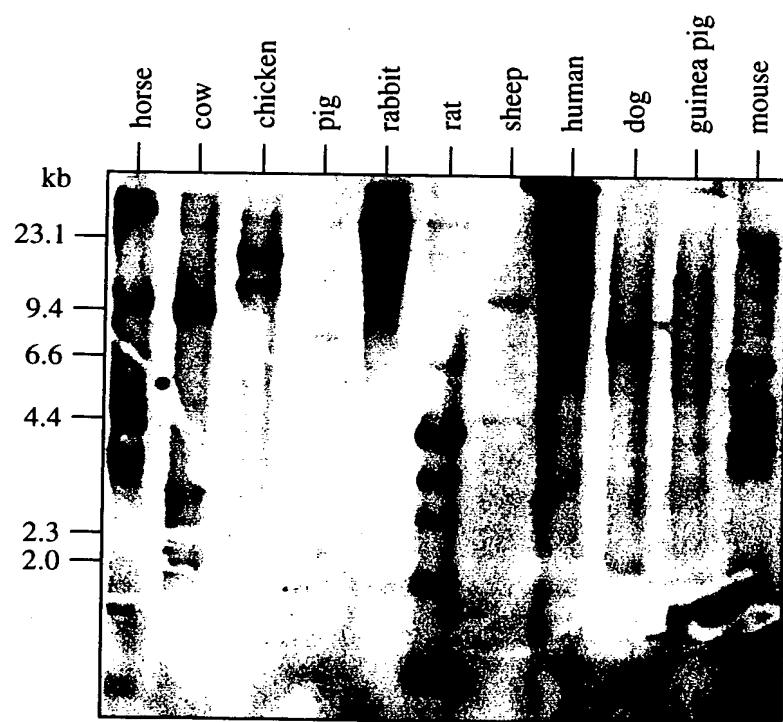


FIGURE 5

FIGURE 6

Cross-species conservation of the NR-4 (IL-13R $\alpha$ ) gene



## FIGURE 7

M	-60	tgaaaagatagaataatggcctcgccgaattcgacggccaggcgagggcgtgcgtc
M	1	ATGGCGCGGCCAGCGCTGCTGGCGAGCTGTTGGTGTGCTACTGTGGACCGCCACCGTG
M	1	M A R P A L L G E L L V L L L W T A T V
H		A P T E T Q P P V T N L S V S V
H		GCGCCTACGGAAACTCAGCCACCTGTGACAAATTGAGTGTCTCTGTT
M	61	GGCCAAGTTGCCGCCACAGAACAGTTCAGCCACCTGTGACGAATTGAGCGCTCTGTC
M	21	G Q V A A A T E V Q P P V T N L S V S V
H		E N L C T V I W T W N P P E G A S S N C
H		GAAAACCTCTGCACAGTAATATGGACATGGAATCCACCGAGGGAGGCCAGCTCAAATTG
M	121	GAAAATCTCTGCACGATAATATGGACGTGGAGTCCTCTGAAAGGAGCCAGTCAAATTG
M	41	E N L C T I I W T W S P P E G A S P N C
H		S L W Y F S H F G D K Q D K K I A P E T
H		AGTCTATGGTATTTAGTCATTTGGCGACAAACAAGATAAGAAAATAGCTCCGGAAACT
M	181	ACTCTCAGATATTTAGTCACTTTGATGACCAACAGGATAAGAAAATGCTCCAGAAACT
M	61	T L R Y F S H F D D Q Q D K K I A P E T
H		R R S I E V P L N E R I C L Q V G S Q C
H		CGTCGTTCAATAGAAGTACCCCTGAATGAGAGGATTTGCTGCAAGTGGGGTCCCAGTGT
M	241	CATCGTAAAGAGGAATTACCCCTGGATGAGAAAATCTGCTGCAAGGTGGCTCTCAGTGT
M	81	H R K E E L P L D E K I C L Q V G S Q C
H		S T N E S E K P S I L V E K C I S P P E
H		AGCACCAATGAGAGTGAGAACGCTAGCATTGGTTGAAAAATGCATCTCACCCCCAGAA
M	301	AGTGCCAAATGAAAGTGAGAACGCTAGCCCTTGGTGAAGAAAGTCATCTCACCCCCGTGAA
M	101	S A N E S E K P S P L V K K C I S P P E
H		G D P E S A V T E L Q C I W H N L S Y M
H		GGTGATCCTGAGTCTGCTGTGACTGAACTTCAATGCATTGGCACAACCTGAGCTACATG
M	361	GGTGATCCTGAGTCCGCTGTGACTGAGCTCAAGTGCATTGGCATAACCTGAGCTATATG
M	121	G D P E S A V T E L K C I W H N L S Y M
H		K C S W L P G R N T S P D T N Y T L Y Y
H		AAGTGTCTGGCTCCCTGGAGGAAGGAATACCAAGTCCGACACTAACTATACTCTACTAT
M	421	AAGTGTCTGGCTCCCTGGAGGAAGGAATACAAGCCCTGACACACACTATACTCTGTACTAT
M	141	K C S W L P G R N T S P D T H Y T L Y Y

**FIGURE 7 (continued...)**

## NR4 expression in mouse tissues

FIGURE 8

